IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Jennie P. **MATHER** *et al.*

Application No.: 10/672,878 — Group Art Unit: 1644

Filed: Sept. 26, 2003 — Examiner: Kim, Yunsoo

For: **COMPOSITIONS AND METHODS FOR GENERATING MONOCLONAL ANTIBODIES REPRESENTATIVE OF A SPECIFIC CELL TYPE**

Commissioner for Patents
Washington, D.C. 20231

Sir:

DECLARATION UNDER 37 C.F.R §1.132

I, Jennie P. Mather, declare as follows:

1. I am a co-inventor of the subject matter described and claimed in the above-referenced patent application, U.S. Patent Application No. 10/672,878 ("the '878 patent application").

2. I have read and understand the non-final Office Action that was mailed January 29, 2007, in the '878 patent application, including the rejection under 35 U.S.C. §103(a). It is my understanding that the basis for this rejection is that the claims are allegedly obvious over a combination of references, none of which teach the production of antibodies using serum-free cells. As I understand, however, the Examiner asserts that it would have been obvious to use such cells to produce monoclonal antibodies were the disclosures of Okabe and US 5,932,704 considered in combination. It is also my understanding that this type of rejection may be rebutted upon a showing of experimental results that would not have been expected upon merely combining the techniques of the two references.

3. By way of illustration, this set of experiments demonstrates that viable and intact cells cultured in serum-free medium are significantly better immunogens than cells cultured in serum-supplemented medium, in generating monoclonal antibodies capable of binding to cell surface antigens representative of the type of cells used for immunization.

4. In this study, adult Schwann cells (rASCs) derived from rat dorsal root ganglia were employed as the immunogens. rASCs were isolated and propagated in serum-free medium according to the procedures detailed in U. S. Patent No. 5,721,139, U. S. Patent No. 5,714,385, and Li, R. (1997) Endocrinology, 138: 2648-2657. Balb/c mice were immunized either intraperitonealy (ip) or via the foot pad (fp) with approximately 5 X 10^6 to 5 X 10^7 intact rASC cells grown in serum-free or serum-supplemented medium.

5. After several rounds of boost immunization, hybridomas were generated by fusing lymphocytes from the mouse spleen (for the group of mice being inoculated interperitoneally) or the mouse lymph nodes (for the group of mice being inoculated at the foot pad) with the myeloma line X63-Ag8.653 using about 35% to about 50% polyethylene glycol 4000 (Oi, V. and Herzenberg, L. (1980)"Immunoglobulin-Producing Hybrid Cell Lines"). An approximately equal number of hybridoma clones was obtained from the two sub-groups of mice being immunized intraperitoneally with rASC cells grown in medium supplemented with or without serum. However, four times more positive hybridoma clones were found to produce monoclonal antibodies directed to surface antigens of rASC cells when serum-free cells were employed as immunogens. As shown in Table 1, whereas only 3% of the hybridomas generated from the mice immunized with serum-cultured rASC cells secrete monoclonal antibodies reactive with intact rASC cells, 12% of the hybridomas obtained from the mice injected with serum-free rASC cells produce monoclonal antibodies exhibiting specific binding to intact rASC cells as determined by FACS analysis (see Example 3 of the specification for detailed experimental procedures concerning FACS). Similarly, a two-fold difference in the percentage of positive hybridoma clones was observed in the group of mice received foot pad immunization with the same immunogens (Table 1). Clearly, cells whose surfaces are free of serum are more effective in generating monoclonal antibodies capable of binding to surface antigens, regardless of the route of inoculation.

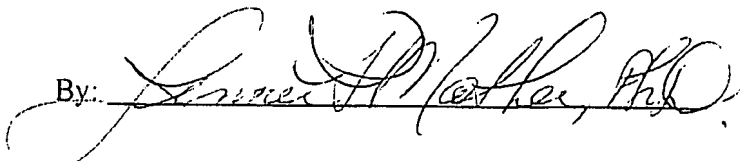
TABLE 1:

Condition	Intraperitoneal immunization with rASC cells cultured in medium supplemented with 10% fetal calf serum (without adjuvant)	Intraperitoneal immunization with rASC cells cultured in serum-free medium (without adjuvant)	Foot pad immunization with rASC cells cultured in medium supplemented with 10% fetal calf serum (without adjuvant)	Foot pad immunization with rASC cells cultured in serum-free medium (without adjuvant)
Total No. of Hybridoma Clones	460	480	64	34
No. of Negative Hybridoma Clones	448	422	63	33
No. of Positive Hybridoma Clones	12	58	1	1
Percentage of Positive Hybridoma Clones	3%	12%	1.5%	3%

(The total number of hybridoma clones represents the sum of hybridoma clones generated by fusing lymphocytes with myeloma cells X63-Ag8.653. Hybridoma clones that secrete monoclonal antibodies capable of binding to intact, unfixed rASC cells as determined by a FACS analysis are denoted "positive hybridoma clones". Conversely, those hybridomas that fail to produce antibodies or that secrete monoclonal antibodies incapable of binding to intact rASC cells in a FACS analysis are categorized as "negative hybridoma clones".)

6. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are

punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

By: 

Dated: July 27, 2007